

MICROBIAL SYNTHESIS OF FOOD FROM COAL-DERIVED MATERIALS

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ABSTRACT

Recent reports on the microbial production of protein from petroleum fractions for use as human or animal food supplements prompted studies on the feasibility of producing microbial protein from coal-derived materials. Growth yields of four species of the yeast *Candida* on several low temperature lignite tar and Fischer-Tropsch synthetic liquid fuel fractions were compared with yields obtained on a normal paraffin fraction derived from petroleum. It was found just as feasible technically to produce microbial food material from low temperature tar and Fischer-Tropsch fractions as from petroleum-derived paraffins. Growth yields were as high as 99.8% (Fischer-Tropsch fraction), 95.2% and 84.2% (two low temperature lignite tar fractions) of the yields obtained on the petroleum-derived paraffin fraction. Similar studies on producing microbial food from coal acids, nitric acid-oxidized anthracite, and a mixture of polynuclear hydrocarbons found in relatively large amounts in high temperature coal tar revealed that these substrates did not support the growth of yeasts. However, a number of bacterial cultures grow at the expense of these materials. Determinations of growth yields are in progress.

INTRODUCTION

The problem of the world's population explosion is further compounded by an overall global food shortage. New or unusual sources of food, especially high quality protein, are needed as animal feed supplements or for human consumption. The Permanent Section on Food Microbiology and Hygiene, International Association of Microbiological Societies, in an unanimous resolution calling for an increased contribution of microbiology to world food supplies, outlined several research areas, including hydrocarbon microbiology, which might lead to increased world food production.^{1/} Recently, reports have appeared dealing with the microbial conversion of petroleum hydrocarbons to protein, vitamins or amino acids. High yields of yeast cells rich in protein and vitamins have been obtained at the expense of the n-alkanes (preferably C₁₀ or higher) in crude petroleum fractions,^{2-6/} feed stocks,^{7,8/} or with the pure hydrocarbons themselves.^{9-11/} Microbial synthesis of amino acids from petroleum products has also been reported.^{12,13/}

The rate of microbial protein synthesis far exceeds the rate at which animals synthesize protein. One 500 kg. cow fed by grazing can synthesize 0.5 kg. of protein per day,^{15/} whereas 500 kg. of microorganisms growing on paraffinic hydrocarbons could synthesize 1250 kg. of protein per day.^{3/} It has been estimated that 3 million tons of protein per year (equal to the world's present protein deficit) could be produced by microorganisms at the expense of only 1% of the world's annual production of 700 million tons of crude paraffinic petroleum.^{3/}

Coal, in addition to petroleum and natural gas, is one of the world's cheapest sources of fixed carbon and energy. The present paper reports the results of our studies on the feasibility of growing microorganisms for their food value at the expense of materials derived from coal.

EXPERIMENTAL

Materials. Three fractions of Bureau of Mines Fischer-Tropsch synthetic liquid fuel (iron catalyst) were used; fraction FTL (boiling range 0° to 204°), fraction FTD (boiling range 204° to 316°), and fraction FTW (boiling range > 316°).

Two fractions of hexane-soluble material from Rockdale lignite low temperature tar were obtained from the Texas Power and Light Company; the hexane solubles forerun (HSF) and the hexane solubles distillate (HSD). The HSF fraction constituted 7% and the HSD fraction 46% of the primary tar. The composition of fractions HSF and HSD are given in table 1. Phenolic compounds were removed by chromatographing fractions HSF and HSD on alumina with petroleum ether as the eluent to yield phenol-free fractions HSF \emptyset and HSD \emptyset . Approximately 20% by weight of starting material was removed by this procedure.

Table 1. Approximate composition of Rockdale lignite low temperature tar fractions, volume percent

Type of constituent	HSF	HSD
Caustic solubles	6-8	10-15
Acid solubles	2-4	1-3
Neutral oil	88-92	80-90
Paraffins	13-15	15-20
Olefins	40-55	40-50
Alpha-olefins	17-20	17-20
Aromatics	30-47	35-45

A paraffin-rich fraction (CTP) and a linear paraffin-olefin fraction (CTPO), both derived from the neutral oil of low temperature tar, were supplied by the Bureau of Mines' Morgantown Coal Research Center. Their analyses are given in table 2. The normal paraffin fraction derived from petroleum (PET) was a product of the Olefins Division of the Union Carbide Corporation. Our mass spectrometric analysis of this fraction is given in table 3.

Table 2. Analyses of paraffin-rich (CTP) and paraffin-olefin (CTPO) fractions from Rockdale lignite low temperature tar, weight percent

Carbon No.	CTP		CTPO	
	n-Paraffin	n-Olefin	n-Paraffin	n-Olefin
C ₈	0.2	--	--	--
C ₉	3.2	--	0.1	0.2
C ₁₀	11.7	0.7	1.3	1.6
C ₁₁	18.2	1.5	3.6	5.3
C ₁₂	22.4	3.3	5.8	7.6
C ₁₃	20.7	2.8	7.8	9.5
C ₁₄	10.8	3.7	7.5	10.9
C ₁₅	0.8	--	6.7	9.4
C ₁₆	--	--	4.6	5.6
C ₁₇	--	--	2.3	2.9
C ₁₈	--	--	4.2	3.1
	88.0	12.0	43.9	56.1

Table 3. Mass spectrometric analysis of n-paraffin fraction (FT) from petroleum

<u>Carbon No.</u>	<u>Volume percent</u>
C ₉	0.5
C ₁₀	7.2
C ₁₁	37.9
C ₁₂	29.1
C ₁₃	23.4
C ₁₄	1.9

Microorganisms. Cultures were obtained from soil by standard enrichment culture techniques or from the culture collections of the University of Pittsburgh, Syracuse University, and the University of Iowa.

Measurement of Growth Yields. Basal medium NX was prepared by adding NH_4NO_3 (5.0 g.), K_2HPO_4 (2.5 g.), and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.0 g.) to one liter of tap water. The pH was adjusted to 7.0 and any insoluble salts were removed by filtration. After sterilization by autoclaving, filter-sterilized yeast extract was added to a final concentration of 0.01%.

Inocula were prepared from cultures grown overnight in 50 ml. of Mycophil broth. The resultant growth was collected by centrifugation, washed twice in the sterile mineral salts solution of medium NX and resuspended in 20 ml. of the same solution. One ml. of washed cell suspension served as standard inoculum for all experiments.

For growth yield studies inocula prepared as above were added to 50 ml. of medium NX in 300-ml. Erlenmeyer flasks in triplicate. A quantity of substrate equivalent to 0.3 ml. was weighed into each flask. Triplicate controls consisted of inoculated flasks without added substrate. Cultures were incubated at 30° C. on a rotary shaker (225 r.p.m.) for six days. The resultant growth was collected on tared 2-inch diameter solvent resistant membrane filters (0.20 μ pore size), washed with 10-ml. volumes of acetone and n-hexane, dried overnight in air, and then weighed. All data are corrected for growth of controls.

RESULTS

More than 200 cultures of bacteria, yeasts, and fungi were screened for their ability to grow on Fischer-Tropsch and low temperature tar fractions. The yeasts Candida lipolytica strains 409, 409A, 409B, and Candida tropicalis strain 410 were selected as the most promising cultures with respect to total cell yield, ability to utilize diverse substrates, and resistance to reasonably high substrate concentrations. These preliminary studies also indicated that growth yields on Fischer-Tropsch fraction FTL and low temperature tar fractions HSF and HSF \emptyset were negligible. No further studies were made with these fractions.

Comparative Growth Studies. Pure Compounds vs. Coal-Derived Material. Absolute growth yields, in mg. dry weight per gram of substrate added, are given in table 4. The highest growth yields for all cultures (ranging from 433 to 719 mg. dry weight) were obtained with n-hexadecane as substrate; C. lipolytica 409 and 409A gave the best yields. Growth yields of 300 mg. or higher were obtained when 1-octadecene (C. lipolytica 409 and C. tropicalis 410) and Fischer-Tropsch fraction FTW (C. lipolytica 409B) served as growth substrates. Other substrates yielding

more than 200 mg. dry weight were paraffin-rich low temperature coal tar fraction CTP (all cultures), and Fischer-Tropsch fractions FTD (*C. lipolytica* 409B) and FTW (*C. lipolytica* 409). All cultures yielded less than 100 mg. dry weight on low temperature tar fraction HSDØ.

Table 4. Growth yields on pure compounds, low temperature tar, and Fischer-Tropsch fractions^{1/}

Substrate	<i>C. lipolytica</i>			<i>C. tropicalis</i>
	409	409A	409B	410
Low temperature tar				
HSDØ ^{2/}	83	43	0	62
CTP	272	276	294	208
Fischer-Tropsch				
FTD	134	115	280	-
FTW	287	127	344	200
Pure compounds				
1-Octadecene	357	295	244	344
n-Hexadecane	719	628	433	453

1/ Average of triplicate cultures. Data in mg. dry weight per gram substrate added, corrected for growth in controls.

2/ Phenols removed by two passes on alumina column.

Figures 1 and 2 illustrate comparative growth yields relative to n-hexadecane using the data in table 4. On all substrates tested, with the exception of low temperature tar fraction HSDØ, *C. lipolytica* 409B consistently gave yields greater than 50% relative to n-hexadecane and appears to be the most versatile culture. *C. tropicalis* 410 is noteworthy for its ability to utilize the terminal olefin 1-octadecene (75% relative to n-hexadecane).

Comparative Growth Studies. Petroleum-Derived Paraffin Fraction vs. Coal-Derived Material. Table 5 compares absolute growth yields on coal-derived material with yields on a normal paraffin fraction derived from petroleum (PET). The three *C. lipolytica* cultures yielded over 300 mg. dry weight on petroleum fraction PET. Yields greater than 300 mg. dry weight were also obtained on Fischer-Tropsch fraction FTW with *C. lipolytica* 409 and 409B. All cultures gave yields greater than 200 mg. dry weight on low temperature tar fraction CTP. *C. lipolytica* 409B and *C. tropicalis* 410 both produced more than 200 mg. of dry cells on low temperature tar fraction CTPO. *C. lipolytica* 409B also yielded more than 200 mg. dry weight on Fischer-Tropsch fraction FTD. All cultures yielded negligible or no growth on low temperature tar fraction HSD; however, upon removal of the phenolic constituents from this fraction, *C. lipolytica* 409B and *C. tropicalis* 410 produced more than 100 mg. of dry cell material.

Figures 3 and 4 illustrate comparative yields relative to petroleum fraction PET using the data in table 5. *C. lipolytica* 409 was outstanding on Fischer-Tropsch fraction FTW and low temperature tar fraction CTP, yielding 99.8% and 84.5%, respectively, of the growth obtained on PET. *C. lipolytica* 409A gave a relative growth yield of 70.5% on fraction CTP. The versatility of *C. lipolytica* 409B is again illustrated by relative yields ranging from 60.4% (fraction CTPO) to 85.3% (fraction FTW) on all substrates except fractions HSD and HSDØ. *C. tropicalis* 410 was outstanding in its relative ability to grow on low temperature tar fractions

CTP (95.2%) and CTPO (84.2%), although absolute yields were below those obtained with *C. lipolytica* 409B on the same substrates (see table 5). Also interesting is the apparent correlation between the abilities of *C. lipolytica* 409B and *C. tropicalis* 410 to use a terminal olefin (table 4) and their ability to grow on low temperature tar fraction CTPO which contains 56% of olefinic compounds (table 2). The *C. lipolytica* 409 and 409A cultures, which gave lower yields on 1-octadecene relative to n-hexadecane (figure 1), reflected this in their lower absolute and relative yields on CTPO compared with CTP (figure 3, table 5).

Table 5. Growth yields on low temperature tar, Fischer-Tropsch, and petroleum fractions^{1/}

Substrate	<i>C. lipolytica</i>			<i>C. tropicalis</i>
	409	409A	409B	410
Low temperature tar				
HSD ^{2/}	14	6	0	0
HSD ^{3/}	73	72	106	102
CTP	259	235	287	232
CTPO	113	124	233	205
Fischer-Tropsch				
FTD	114	150	243	0
FTW	306	167	329	161
Petroleum				
PET	307	333	385	244

1/ Average of triplicate cultures. Data in mg. dry weight per gram substrate added, corrected for growth in controls.

2/ Phenols not removed.

3/ Phenols removed by three passes on alumina column.

Growth Studies on Other Coal-Derived Materials. Studies were begun to investigate the feasibility of producing microbial food from other materials derived from coal, such as acids obtained by oxidizing coal with air, nitric acid-oxidized anthracite, and a mixture of polynuclear hydrocarbons found in high temperature coal tar.

A 56% aqueous solution of coal acids (obtained from the Dow Chemical Co.) and a water-soluble mixture of aromatic acids from the alkaline oxidation of coal (obtained from the Carnegie Institute of Technology) were tested as growth substrates for all of our yeast cultures. No growth was obtained. Similarly, soil enrichment culture procedures designed to favor the isolation of yeasts gave negative results. However, a number of bacterial cultures capable of growing on the coal acids were isolated; quantitative studies on growth yields are in progress.

A series of oxidation products from the nitric acid oxidation of anthracite was obtained from the Bureau of Mines Anthracite Research Center.^{14/} The acid-soluble residue of the 1000-hour oxidation time supports the growth of a bacterial culture previously isolated on one of the coal acids. Growth yield studies and enrichment culture procedures are in progress.

A mixture of polynuclear hydrocarbons found in relatively large amounts in high temperature coal tar was prepared. It was composed of (by weight percent) 1-methylnaphthalene, 59.2; 2-methylnaphthalene, 16.3; naphthalene, 11.9; phenanthrene, 12.5. A number of bacterial cultures, but no yeasts, capable of growing on this material have been isolated from soil. Growth yield studies are in progress.

DISCUSSION

In practice, over 80% conversion of hydrocarbon substrates to cell material has been obtained.^{3,6,9-11/} Although our absolute growth yields on pure hydrocarbons, petroleum, Fischer-Tropsch, and low temperature tar fractions were somewhat lower, it must be remembered that our experiments were designed solely to test the feasibility of using these substrates for microbial food production. Improvements in the nutritional quality of the growth medium, closer control of pH, more efficient aeration, and other practices of the fermentation microbiologist's art will undoubtedly result in higher cell yields.

It is clear that some of the substrates derived from coal which were tested are closely comparable to petroleum-derived normal paraffins in their ability to support microbial growth with the concomitant production of cell material (food). Paraffin-rich low temperature tar fraction CTP and Fischer-Tropsch fraction FTW were outstanding in this regard.

Oxidized coals and polynuclear aromatic hydrocarbons may be suitable for the growth of bacteria, rather than yeasts, for use as food material. Further experiments along this line are in progress.

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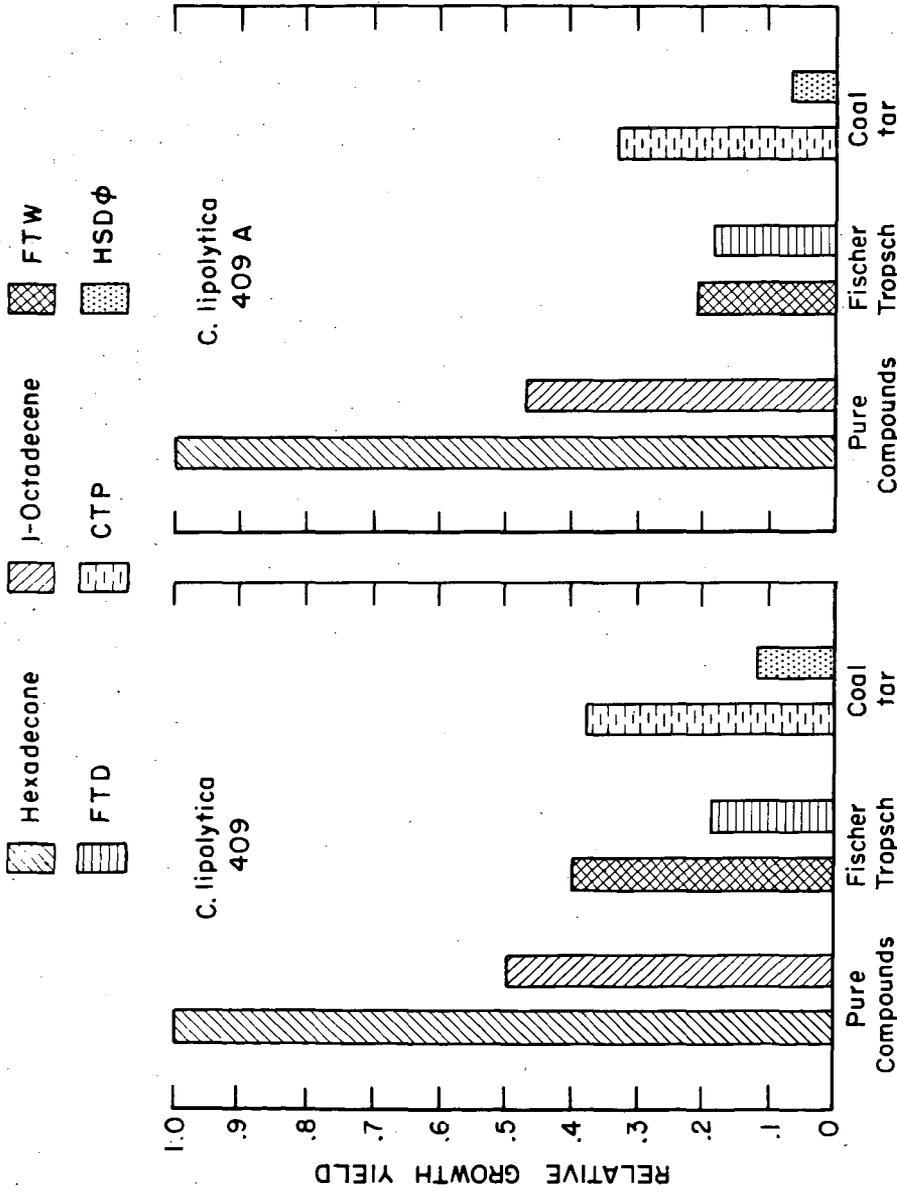


Figure 1.- Growth yields relative to n-hexadecane.

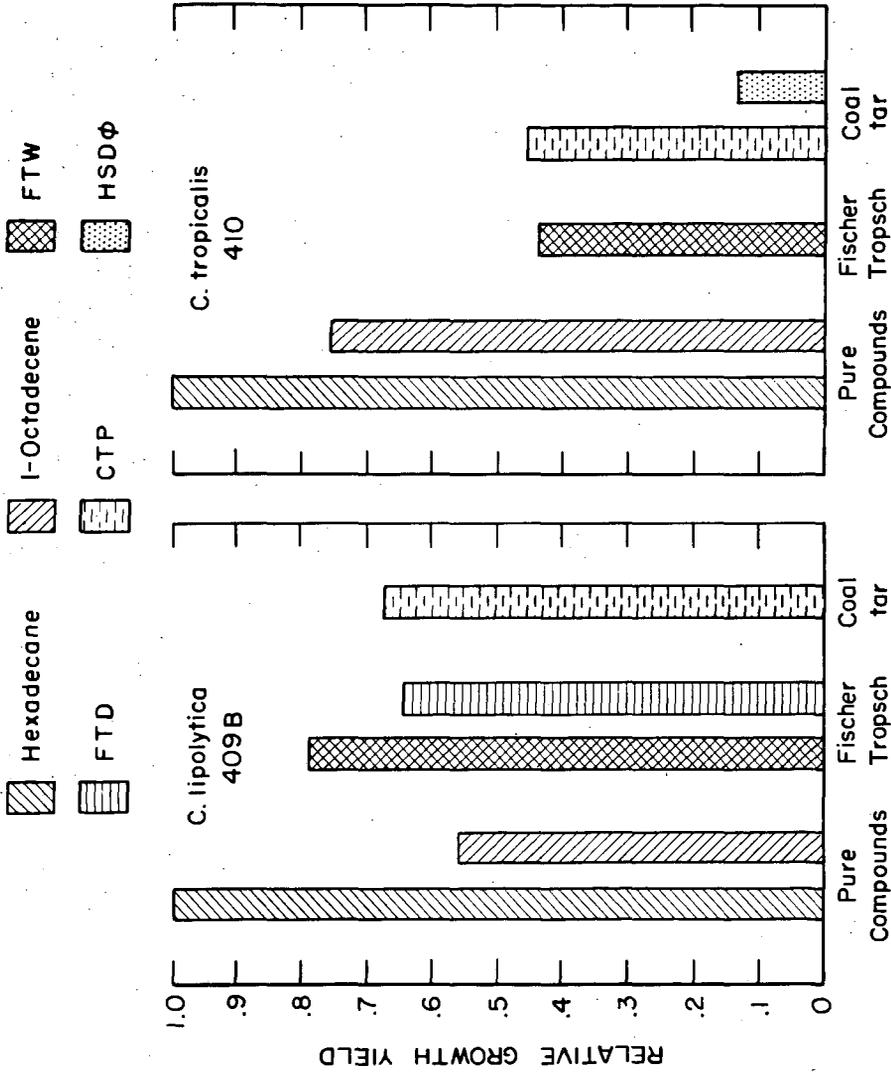


Figure 2.-Growth yields relative to n-hexadecane.

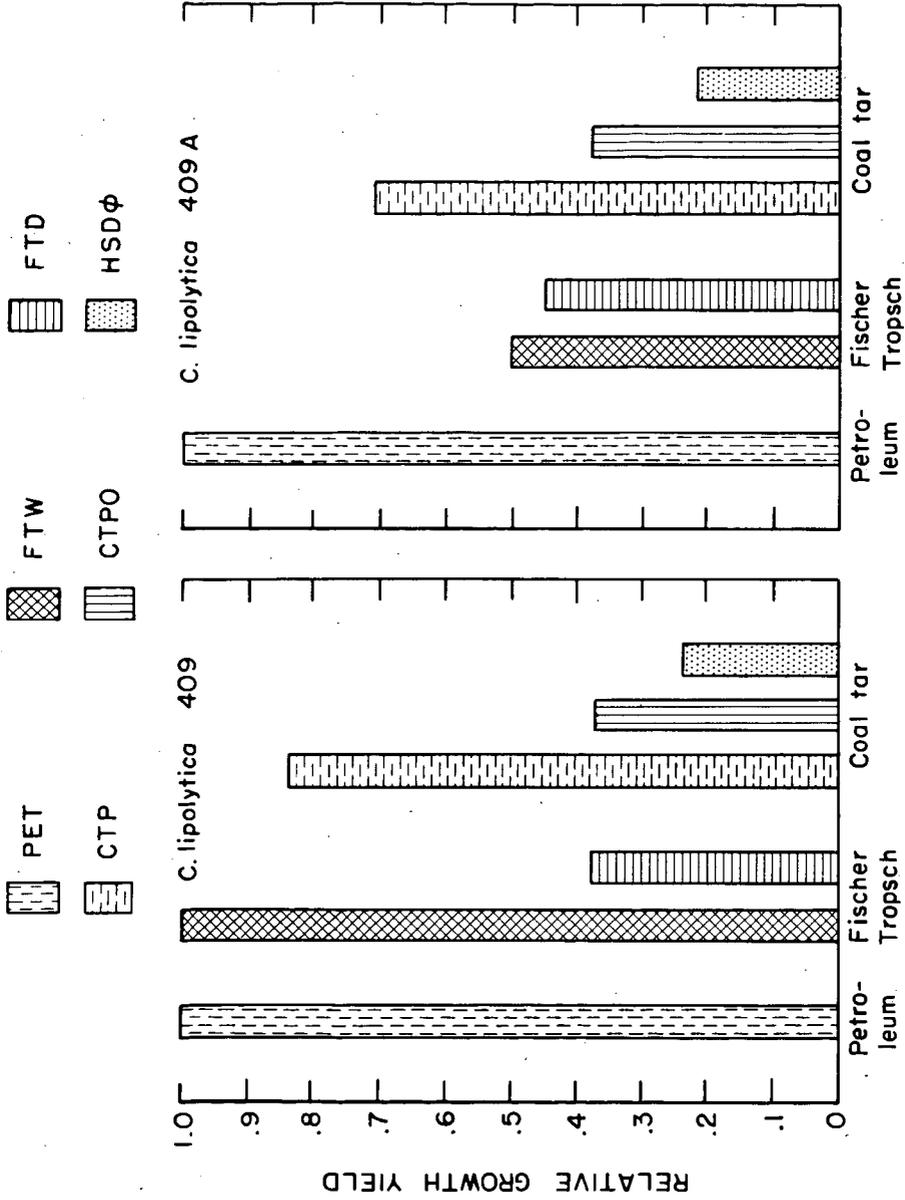


Figure 3.- Growth yields relative to petroleum.

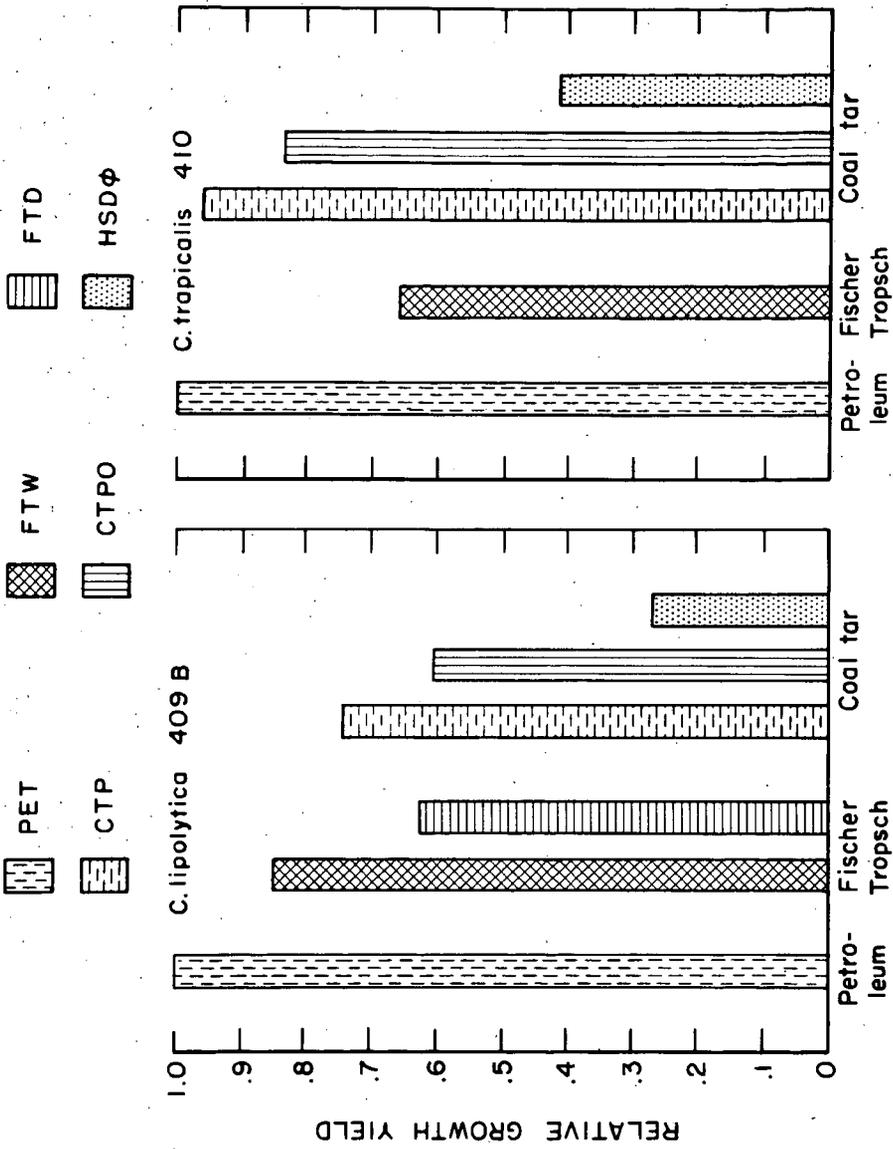


Figure 4.-Growth yields relative to petroleum.